A Histochemical Study on some Dehydrogenases During the Estrus Cycle, Pseudopregnancy and Decidualization in the rat uterus

Tohoku Journal of Agricultural Research
Vol. 21, No. 2, June 1970
Printed in Japan

A Histochemical Study on some Dehydrogenases During the Estrus Cycle, Pseudopregnancy and Decidualization in the rat uterus

Jutaro TAKAHASHI, Tadahiko HOSHINO and Saburo TAKEUCHI.

Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai, Japan
(Received May 11, 1970)

Summary

Glycogen and several dehydrogenases related to carbohydrate metabolism in the rat uterus were studied histochemically during the estrus cycle, pseudopregnancy and decidualization. The activity of four enzymes, (SDH, LDH, NADDH and G6PDH) in various regions, except for muscle layer, was well correlated with the deposition of glycogen at each stage. The deposition of glycogen and of these enzymes has indicated cyclical changes in localization and intensity during the estrus cycle. On the 4th day of pseudopregnancy, these four enzymes showed a comparatively higher activity in the epithelium, subepithelial layer and uterine gland.

It is suggested that a higher activity of these enzymes on the 4th day of pseudopregnancy is related to the estrogen surge on the 3rd day of pseudopregnancy.

The changes of glycogen and enzymes related to carbohydrate metabolism have been demonstrated histochemically (1～4) and biochemically (5～7) in the rat uterus. Recently, more extensive studies have been made in the rat (8,9) and the mouse (10～13). However, little is yet known about the timecourse changes of dehydrogenases related to glycogen metabolism during the estrus cycle, pseudopregnancy and decidualization. Accordingly, histochemical studies on glycogen and on some dehydrogenases related to carbohydrate metabolism in the rat uterus during estrus cycle, pseudopregnancy and decidualization have been made.

Materials and Methods

Mature virgin female rats of the Wistar strain, weighing 180–290 g, were housed in an air conditioned room (22±1°C). The light was automatically turned on at 7 A.M. and off at 7 P.M..A Vaginal smear of all animals was recorded prior to and throughout the course of all experiment. Only animals having three times

or more regular estrus cycles prior to the start of the investigation were selected.

Preparations of Animals

a) Normal cycle; Four animals were taken for each stage of the estrus cycle — proestrus, estrus, metestrus and diestrus. They were examined at 9:00 A.M. Then, they were killed at 10:00 A.M. on the same day.

b) Pseudopregnancy; Pseudopregnancy was induced by faradic stimulation of cervix uteri at proestrus and estrus. (14) The first day on which the vaginal smear became leucocytic after stimulation was noted as day 1 of pseudopregnancy. Four animals were taken for each day from day 1 to day 4 of pseudopregnancy.

c) Formation of deciduoma; Laparotomy was performed on day 4 of pseudopregnancy and into the right horn was injected 10 μl of sesame oil through the utero-tubal junction under ether anaesthesia. Two animals were killed daily from Day 5 to Day 8 of pseudopregnancy. Left horn was left intact in pseudopregnancy.

PAS Positive Substance and Glycogen

The animals were killed by a blow on the head and the uterine horns were rapidly removed. The horns were fixed in Carnoy's fluid, embedded in paraffin and sectioned at 10 μ for the demonstration of glycogen.

The sections were stained by the Periodic acid Schiff reaction (PAS) (15) with or without pretreatment for 1 hour at 37°C with saliva.

Dehydrogenases

The enzymes were examined as follows:

(1) For Succinate dehydrogenase (SDH), Lactate dehydrogenase (LDH), NADH dehydrogenase (NADDH), which are concerned with carbohydrate metabolism.

(2) For Glucose-6-phosphate dehydrogenase (G6PDH, which is a gateway to the pentose shunt and is concerned with the production of energy.)

The blocks of tissue were embedded in egg albumen and fixed in the carbon dioxide-acetone, and then sectioned at 10 μ with the cryostat and stained by the Barka and Anderson method (16) for SDH, LDH and NADDH activity testing, and by the Rudolph and Klein method (17) for G6PDH activity testing. Nito-blue tetrazolium (NBT) salt was used as the electron accepter for all enzymes. The sections were incubated at 37°C for 1 hour except for NADDH, for which the incubation period was 30 min. Control sections were incubated at the same time in a solution without substrate. The middle third of the uterus was used for experiment and the section was divided into layers as shown in Fig. 1. PAS positive substance and enzyme activities were observed under the light microscope and indicated in grades from + to ### for each layer of the uterus.

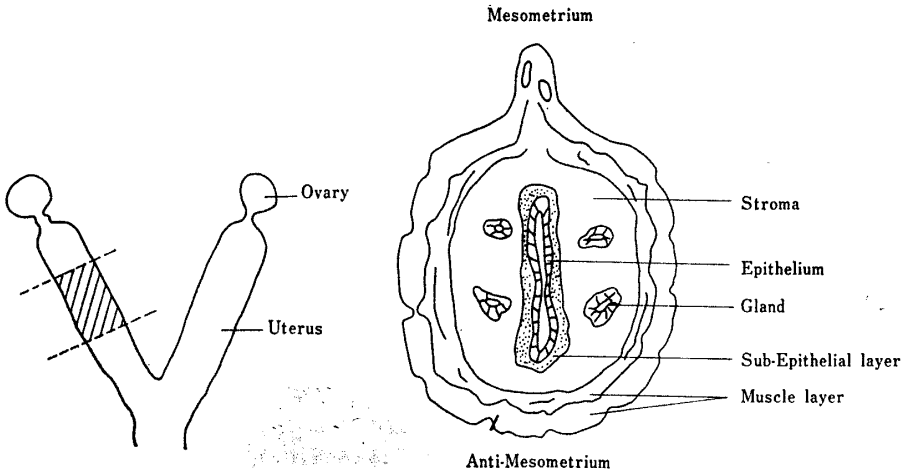


FIG. 1. Diagram to show the portion for experiment and structure of the rat uterus.

Results

PAS Positive Substance and Glycogen

The change of PAS positive substance obtained here is shown in Fig. 2. The glycogen disappearance, by the salivary test, occurs in the muscle layer, but not in the epithelium at the proestrus. On the contrary, the glycogen-bearing cells appear in the epithelium, not in the muscle layer at the metestrus. In the 4th day of pseudopregnancy, the glycogen bearing cells appear in the epithelium, the sub-epithelial layer and in the uterine gland. However, the change of PAS positive substance is not observed in the stroma nor the muscle layer. After the oil injection into the uterus in the 4th day of pseudopregnancy, a deciduomal reaction occurred in the endometrium, especially in the sub-epithelial layer. The glycogen deposition increased in the deciduoma cells and the muscle layer with the advance of deciduoma formation.

Dehydrogenases

The changes of SDH activity in various stages is shown in Fig. 3. The SDH activity is comparatively low in each layer at the proestrus, but tend to increase in the stroma and the sub-epithelial layer at estrus. The SDH of macrophage is especially high. SDH is active in the sub-epithelial layer from the 3rd to the 5th day, while it is active in uterine gland on the 4th day of pseudopregnancy. SDH activity increased in the decidual cells in accordance with the progress of deciduoma formation and is maximum on the 8th day of pseudopregnancy. LDH is active in the epithelium, the subepithelial layer, the uterine gland and the muscle layer at estrus and in the subepithelial layer on the 3rd and 4th day of pseudopregnancy. (Fig. 4). In the site of deciduoma, LDH is strongly active on the 8th day of pseudopregnancy. As shown in Fig. 5, the enzyme of

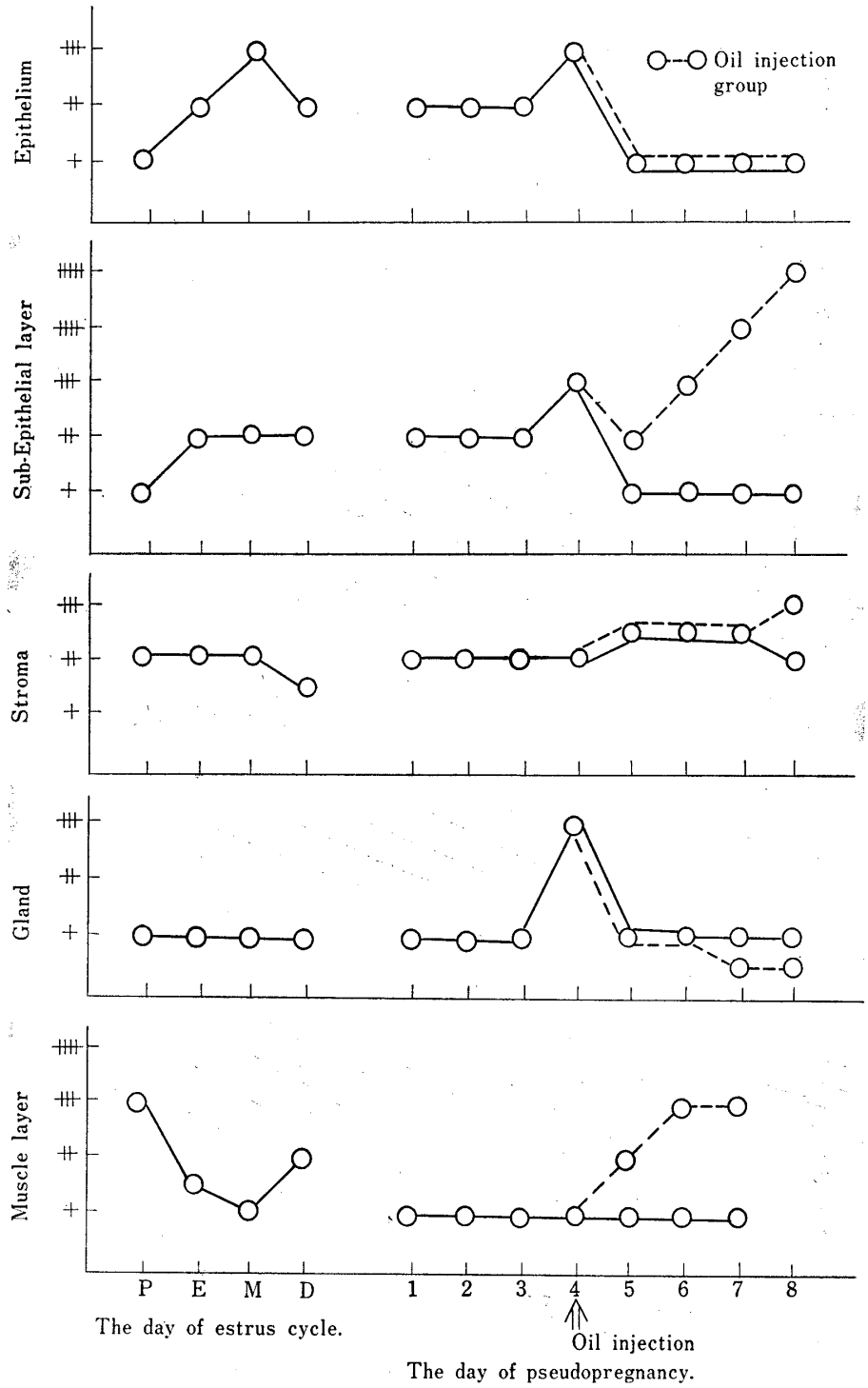


FIG. 12. The changes of PAS positive substance in the rat uterus during estrus cycle, pseudopregnancy and decidualization.
P: Proestrus E: Estrus M: Metestrus D: Diestrus

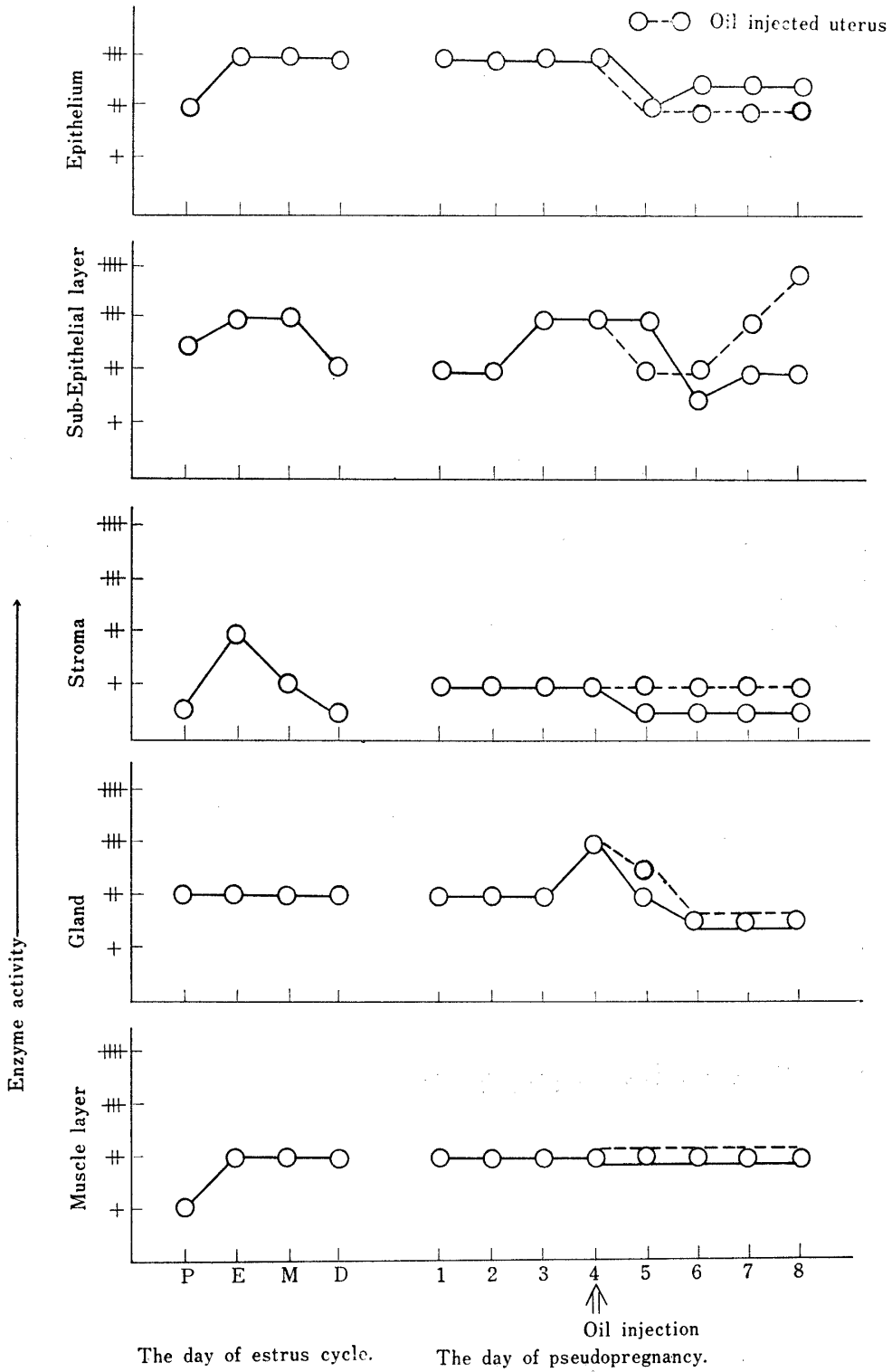


FIG. 3. SDH activity of the rat uterus during estrus cycle, pseudopregnancy and decidualization.

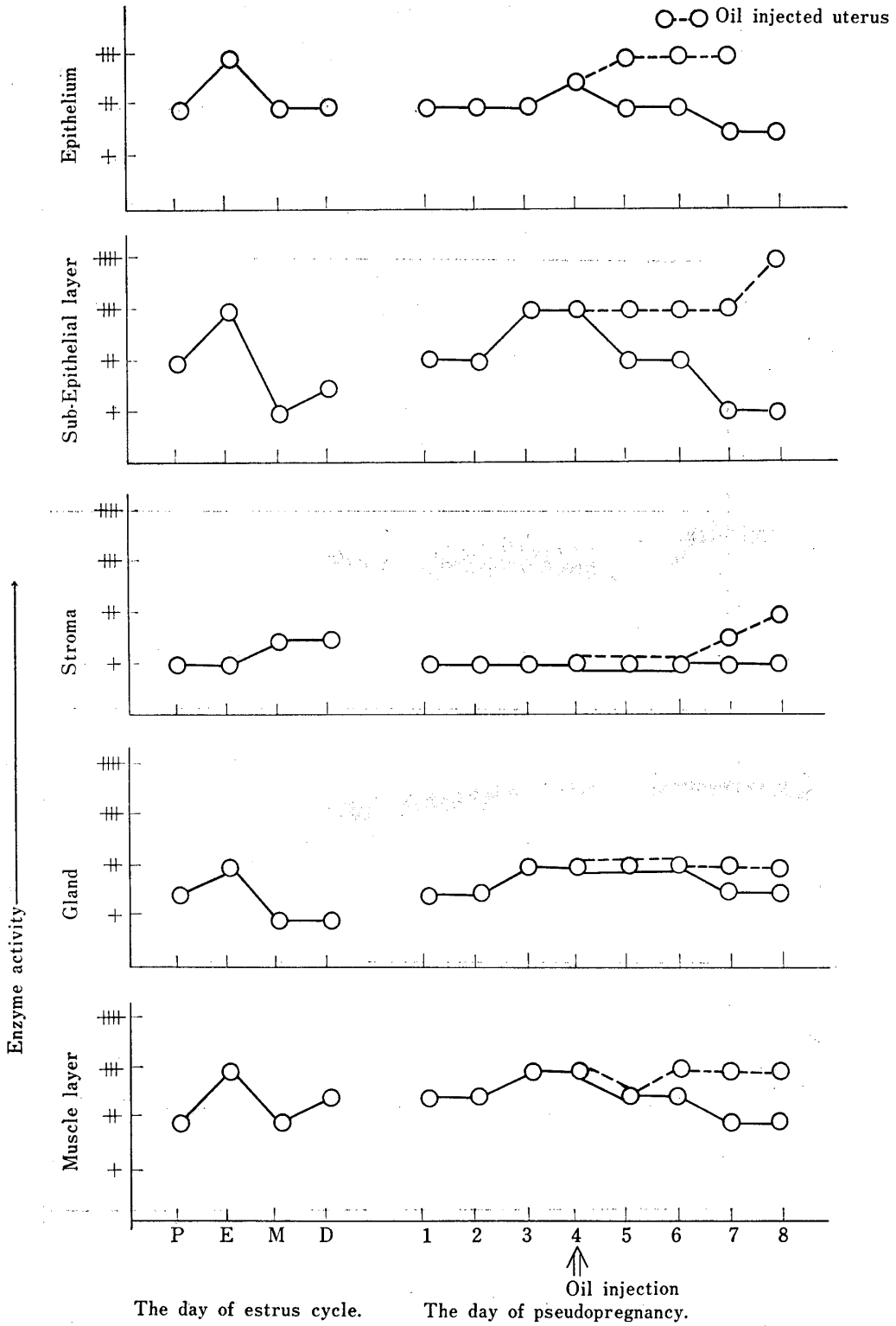


FIG. 4. LDH activity of the rat uterus during estrus cycle, pseudopregnancy and decidualization.

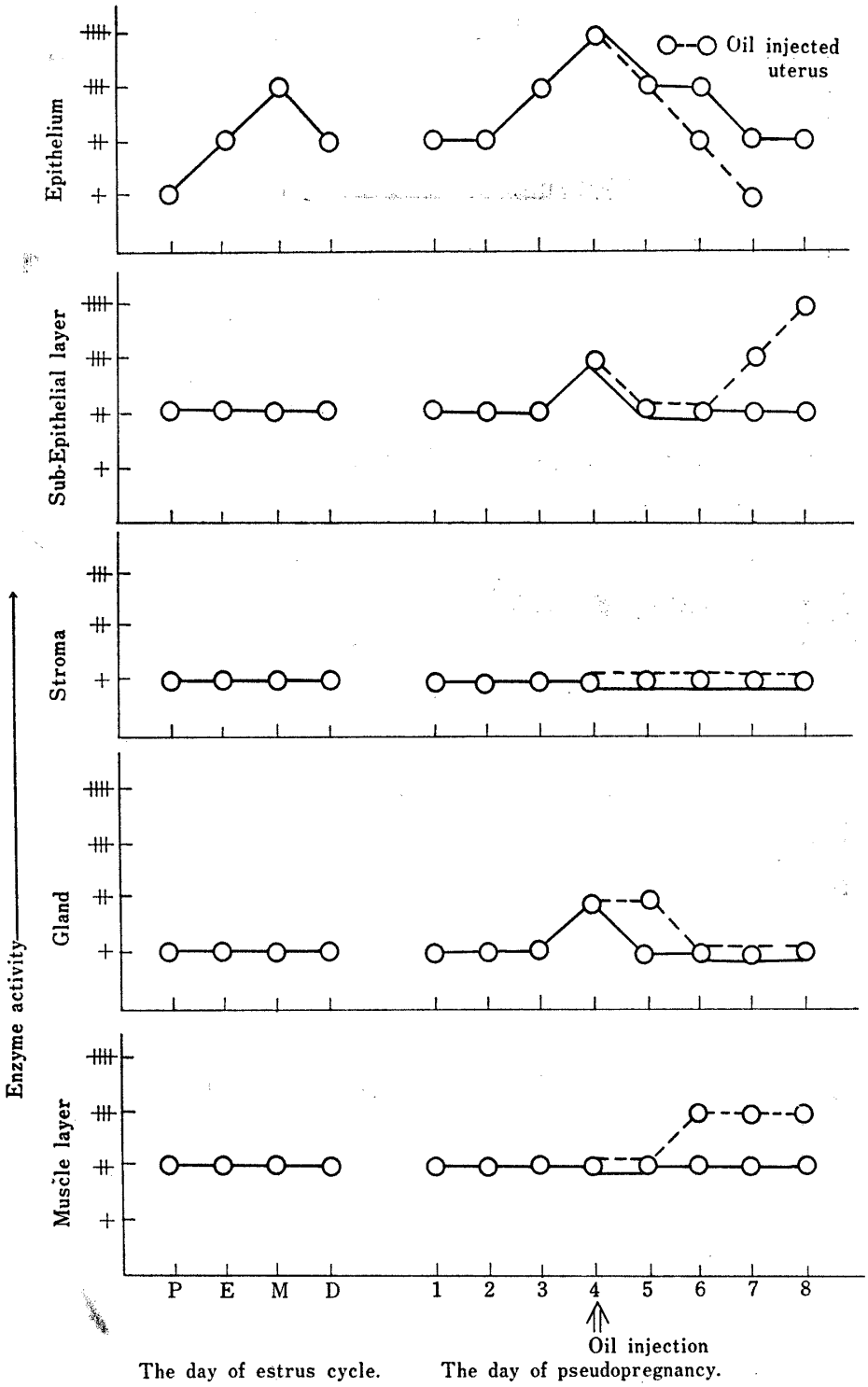


FIG. 5. NADDH activity of the rat uterus during estrus cycle, pseudopregnancy and decidualization.

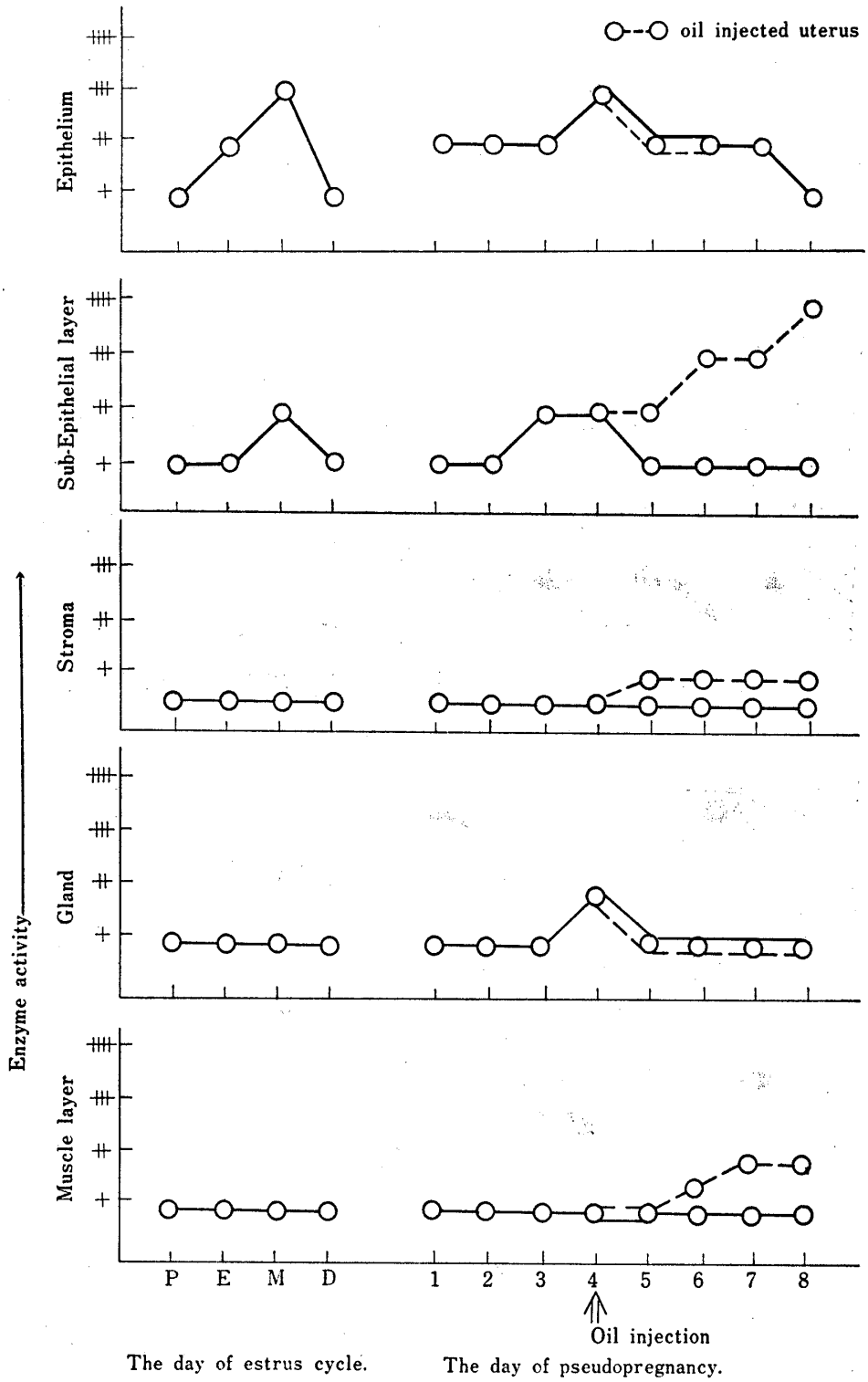


FIG. 6. G6PDH activity of the rat uterus during estrus cycle, pseudopregnancy and decidualization.

NADDH is active in the epithelium at metestrus and in the epithelium, sub-epithelial layer and uterine gland on the 4th day of pseudopregnancy. NADDH activity increased in decidual cells which appear in the sub-epithelial layer after oil injection into the uterus. As shown in Fig. 6, the change of G6PDH activity resembles closely the NADDH activity.

Discussion

PAS Positive Substance and Glycogen

Glycogen deposition shows the cyclical variations in localization and intensity in estrus cycle. The deposition of glycogen occurs in the muscle layer of the uterus and is maximum at proestrus, while the glycogen in the epithelium and sub-epithelial layer is maximum at metestrus. These results are in agreement with the report by Rosenbaum and Goolsby (3). On the 4th day of pseudopregnancy, glycogen bearing cells are observed in the epithelium, sub-epithelial layer and uterine gland. After oil injection into the uterus on the 4th day of pseudopregnancy, glycogen deposition increased in the site of the deciduoma and the muscle layer with the advance of the decidual reaction. In considering such an observation, it seems that this glycogen may be utilized as an energy source at implantation, which takes place within four to five days of gestation. It is probable that the localization of glycogen in both the muscle layer and endometrium is due to a physiological response to the stimulation of hormone secreted from the ovary.

Dehydrogenases

The relations between these oxydative enzymes in the uterus and ovarian hormones have been discussed by many previous workers. The cyclical changes in the activity of SDH in the endometrium examined in the rat histochemically (18～21) and biochemically (5) support the theory that the activity of this enzyme is an index of the functional status of the epithelial cells (22). Bever et al (23) have reported that the administration of estradiol-17β, estrone and estriol increase significantly the specific activity of the lactic dehydrogenase-DPNH oxidase system in uteri of ovariectomized rats. It has been reported that the activity of G6PDH decrease after castration and increase after estradiol administration in the rat uterus (6). The activity of these enzymes has shown cyclical variations in localization and intensity during the estrus cycle, which is almost in agreement with the work of Tiery and Willighagen (21) and then have apparently been influenced by hormones related to the function of the ovary. On the 4th day of pseudopregnancy, these four enzymes have indicated a higher activity in the epithelium, sub-epithelial layer and uterine gland. Shelesnyak and Kraicer (24) have reported that a surge of estrogen from the ovary occurs on the third day of pseudopregnancy.

Then the present results seem to support the hypothesis that the uterus reacting maximally to the stimulus to decidualize on the 4th day of pseudopregnancy is related to the metabolic activity observed in the uterus on the 4th day caused by the estrogen surge (6). As indicated by the relation between these enzymes and glycogen deposition, it is very interesting that the distribution of these dehydrogenases in various regions except for the muscle layer at each stage of the estrus cycle and pseudopregnancy is well correlated to the distribution of glycogen. After the stimulation of the uterus, the activities of these enzymes increased in the decidual cells, especially in the anti-mesometrium rather than the mesometrium of the uterus. Thus, it is suggested that the metabolism of decidual cells are very active, and that the intense activity of these emzymes of deciduoma in the anti-mesometrial region is correlated to the report that the decidual cell reaction occurrs antimesometrially, no matter in what region of the uterus the stimulus is applied (1,25).

References

1) Krehbeil, R.H., *Physiol. Zool.*, **10**, 212 (1937)
2) Bridgmann, J., *J. Morph.*, **83**, 195 (1948)
3) Rosenbaum, R.M., and Goolsby, C.M., *J. Histochem. Cytochem.*, **5**, 33 (1957)
4) Lobel, B.L., Tic, L., and Shelesnyak, M.C., *Acta Endocr.*, **50**, 452 (1965)
5) Bever, A.T., Velardo, J.T., Telfer, M.A., Hisaw, F.L., and Goolsby, C.M., *Fed. Proc.*, **13**, 13 (1954)
6) Eckstein, B., and Villee, C.A., *Endocrinol.* **78**, 409 (1966)
7) Gregoire, A.T., Ramsey, H., and Adams, A., *J. Reprod. Fert.*, **14**, 231 (1967)
8) Christie, G.A., *J. Reprod. Fert.*, **12**, 279 (1966)
9) Christie, G.A., *J. Reprod. Fert.*, **13**, 281 (1967)
10) Finn, C.A., and McLaren, A., *J. Reprod. Fert.*, **13**, 259 (1967)
11) Smith, L.J., *Am. J. Anat.*, **119**, 1 (1966 a)
12) Smith, L.J., *Am. J. Anat.*, **119**, 15 (1966 b)
13) Wong, Y.C., and Dickson, A.D., *J. Anat.*, **105**, 3, 547 (1969)
14) Sugawara, S., and Takeuchi, S., *Jap. J. Zootech. Sci.*, **35**, 80 (1964) (in Japanese, with Englich summary)
15) McManus, J.F.A., *Stain Tech.*, **23**, 99 (1948)
16) Barka, T., and Anderson, P.J., "*Histochemistry; Theory, Practice and Bibliography*", New York, Evanston and London (1963)
17) Rudolph, G., and Klein, H.J., *Histochem.*, **4**, 248 (1964)
18) Rosa, C.G., *Proc. Soc. Exp. Biol. Med.*, (N.Y.), **102**, 198 (1959)
19) Rosa, C.G., and Velardo, J.T., *Ann. N.Y. Acad. Sci.*, **83**, 122 (1959 a)
20) Rosa, C.G., and Velardo, J.T., *Ann. N.Y. Acad. Sci.*, **75**, 491 (1959 b)
21) Tiery, M., and Willighagen, R.G.J., *Anat. Rec.*, **146**, 263 (1963)
22) Foraker, A.G., Celi, P.A., and Denham, S.W., *Cancer*, **7**, 100 (1954)
23) Bever, A.T., Velardo, J.T., and Hisaw, F.L., *Endocrinol.* **58**, 512 (1956)
24) Shelesnyak, M.C., and Kraicer, P.F., "*Delayed Implantation*". ed. by Enders, A.C., Chicago and London (1963).
25) Selye, H., and Mckeown, T., *Proc. Roy. Soc. B.*, **119**, 1 (1935)

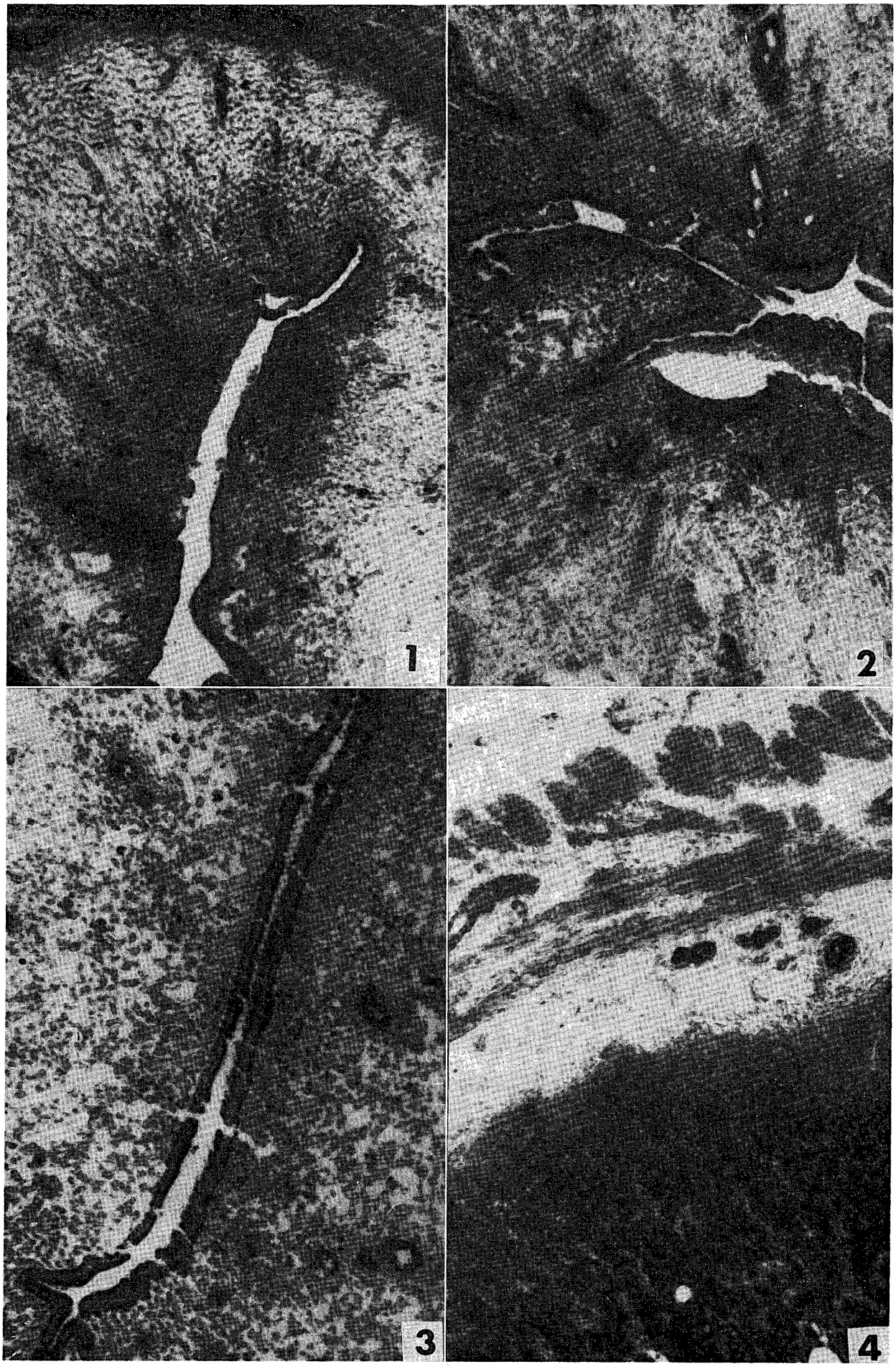
Explanation of Plate.

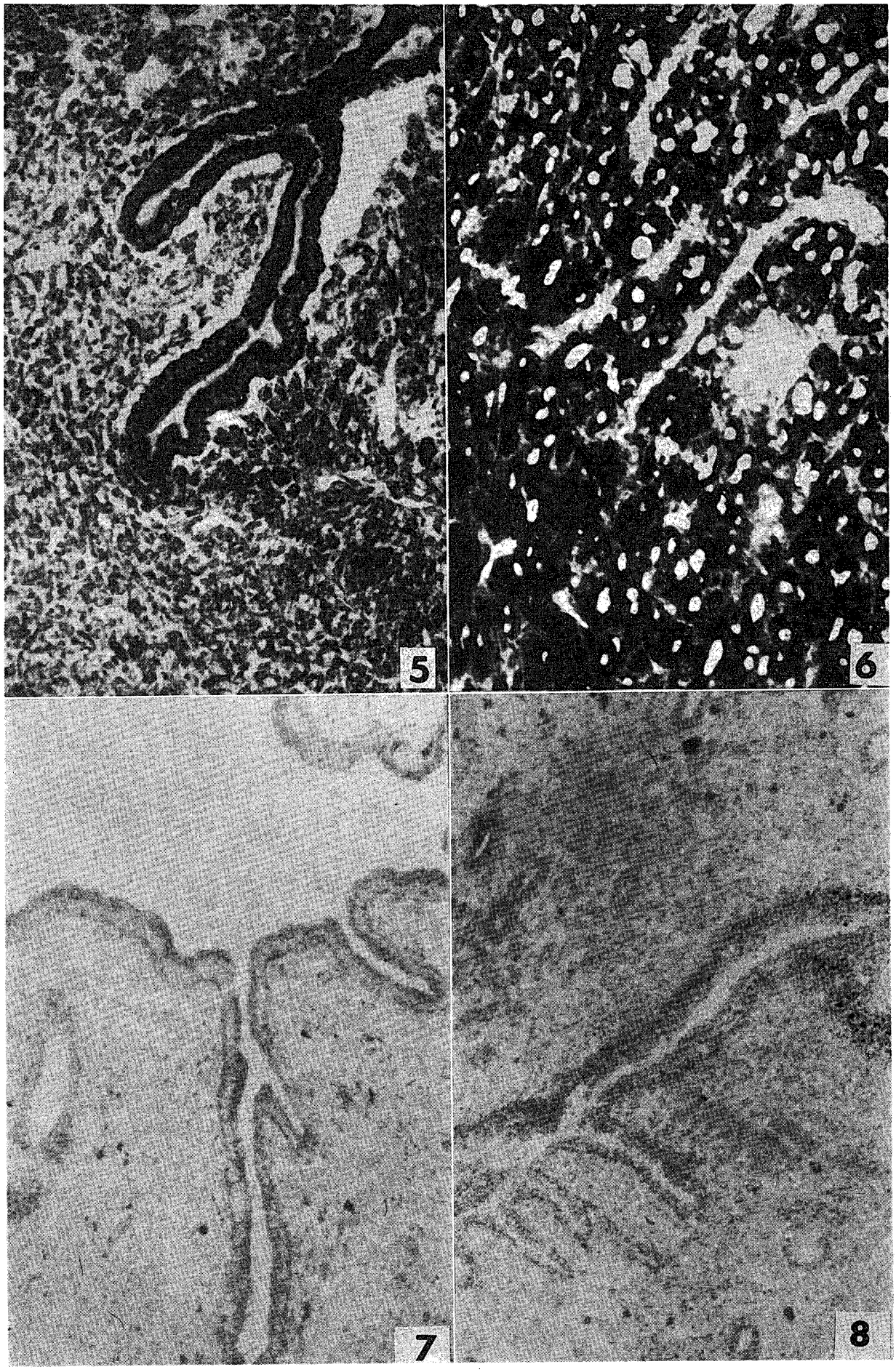
FIG. 1. SDH activity in epithelium and sub-epithelial layer at metestrus. ×100

FIG. 2. High SDH activity in epithelium and sub-epithelial layer on the 4th day of pseudopregnancy. ×100

FIG. 3. SDH activity after 24 hours of oil injection into the uterus. ×100

FIG. 4. SDH activity after 4 days of oil injection into the uterus high SDH enzyme activity is seen in the site of deciduoma. ×40


1
2
3
4


5
6
7
8

Explanation of Plate.

FIG. 5. LDH activity in the 2nd day after uterus stimulation. Strong activity is seen in epithelium and sub-epithelial layer comparatively. ×100

FIG. 6. LDH activity in the 4th day after oil injection into the uterus. Decidual cells show very strong LDH activity. ×400

FIG. 7. G6PDH activity in proestrus. ×100

FIG. 8. G6PDH activity in the 4th day of pseudopregnancy. ×100